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TITLE: **Role of diet modulation and AMPK in ovarian cancer progression and outcome.**

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CONTRACTING ORGANIZATION: **Henry Ford Health System**, Detroit, MI

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14. ABSTRACT

Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States. Given the ambiguity concerning the etiology, difficulties in early detection, absence of any markers, low survival rates and the overall obscurity related with ovarian cancer, more research is needed to identify factors and approaches that could improve ovarian cancer disease initiation, progression and disease free survival. One of these factors which have been largely unexplored is the influence of diet and the metabolic state of the patients. Unfortunately, the role of dietary factors in ovarian cancer prognosis is largely unknown. Particularly, no attention has been given to the metabolic state of a cancer cell and how this state can be modulated by calorie restriction. To determine the effect of diet on ovarian cancer, C57B6 mice were subjected to three types of diet: regular diet (RD), high-energy diet (HED) and calorie-restricted diet (CRD).. Post 30 days of diet, 5×10^6 ID8 mouse ovarian cancer cells were injected in the intra-peritoneal cavity and mice were sacrificed after 60 days, followed by tumor burden evaluation and physiological parameters. A set of mice were treated with metformin Compared to RD and CRD, HED fed mice showed the most extensive tumor nodule formation and the highest tumor score (diaphragm, peritoneum, bowel, liver, kidney, spleen) with higher levels of insulin and leptin in both ascites and serum compared to RD and CRD. The cytokines, MCP-1, VEGF and IL-6, were also higher in the serum and ascites of HED mice. On the other hand, CRD fed mice exhibited a notably reduced tumor burden at every examined site compared to RD and HED mice. This was associated with a significant reduction in levels of insulin, IGF-1, leptin, MCP-1, VEGF and IL-6 both in serum and ascites, compared to RD or HED fed mice. IHC showed tumors from CRD mice to have an increased expression of p-ACC and a lower expression of p-mTOR and p-Akt, compared to RD and HED fed mice, indicating activation of the AMPK pathway. The use of metformin in RD and HED mice resulted in a significant reduction in tumor burden in the peritoneum, liver, kidney, spleen and bowel. Overall, ovarian cancer growth and metastasis occur more aggressively under high-energy diet conditions, while they are significantly curtailed under calorie restriction. CRD is associated with decreased secretion of growth factors and cytokines and activation of AMPK pathway. Metformin seems to inhibit ovarian cancer growth irrespective of caloric intake.   Based on these findings, it is worthwhile to investigate the impact of diet modulation as adjunct to other anticancer therapies in the treatment of epithelial ovarian cancer

15. SUBJECT TERMS

AMPK, ovarian cancer; high fat diet; calorie restricted diet, metformin

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INTRODUCTION

Ovarian cancer is the leading cause of death from gynecologic malignancies in the United States (1). Given the ambiguity concerning the etiology, difficulties in early detection, absence of any markers, low survival rates and the overall obscurity related with ovarian cancer, more research is needed to identify factors and approaches that could improve ovarian cancer disease initiation, progression and disease free survival.

One of these factors which have been largely unexplored is the influence of diet and the metabolic state of the patients. Unfortunately, the role of dietary factors in ovarian cancer prognosis is largely unknown. There are no conclusive guidelines regarding ovarian cancer and food/nutrient intake or physical activity or obesity. There are conflicting reports on this topic that needs further evaluation (2-4). Particularly, no attention has been given to the metabolic state of a cancer cell and how this state can be modulated by calorie restriction. One of the main culprits of metabolic dysfunction is the IGF-Insulin pathway that has been shown to play a major role in ovarian cancer progression and contributes to the metabolic syndrome. Increased levels of insulin and insulin growth factor signaling and increased pro-inflammatory mediators are found in ovarian cancer too. In general, obesity and high caloric intake are known as exacerbating factors in the progression of various cancers including breast and endometrial cancer, while calorie restriction is an ameliorating factor (5-8). However, no relationship has been established between diet, energy metabolism and ovarian cancer. Recently some studies have suggested that low-fat dietary pattern may reduce the incidence of ovarian cancer. High energy and calorie restricted diet affects the overall energy metabolism in body which impacts and in turn gets modulated by AMP-activated protein kinase (AMPK).

AMPK is a highly conserved hetero-trimeric serine/threonine protein kinase that acts as an ultra-sensitive cellular energy sensor maintaining the energy balance within the cell (9). Recent studies have identified AMPK activation as having an anti-proliferative effect on cells via regulation of mainly protein translational (mTOR) and lipid biosynthetic pathways to restrict cell growth (10).

This study is designed to understand the role of energy metabolism using nature of diet (high vs low energy) focusing on AMPK as a central energy regulator in ovarian cancer progression using a syngenic mice model. We are proposing a novel **hypothesis** that *low calorie diet will activate AMPK and thereby shifting the energy balance leading to improved outcome in patients with ovarian cancer.* To explore this hypothesis, we had proposed the following specific aims.

Aim 1: **To investigate if diet modulation influences ovarian cancer progression via AMPK in C57B6 mouse model using ID8 mouse ovarian epithelial cancer cells.**

Aim 2: **Therapeutic potential of AMPK activators as diet supplement to enhance energy metabolism in regulating metabolic derangement of tumor progression.**

The goal of this study is to evaluate a link between nature of food intake (energy-wise) and ovarian cancer which may address how dietary interventions may affect energy metabolism and modulate ovarian cancer outcome.

Due to the change of institute for the PI and time required to set a new lab from scratch, the work is currently behind the proposed SOW. We have completed all the mouse studies and are presently characterizing the tumor tissues and bio-fluids from the last experiment.

We will be also asking for a no-cost extension to complete the pending work.

TIME LINE OF EVENTS:

The grant was awarded to the PI, Ramandeep Rattan, while at Mayo Clinic, Rochester MN 55905 in 2010. By the time all the paperwork involved was completed the PI had moved to Henry Ford Health System (HFHS), Detroit, MI 48202 in September of 2011. The grant was transferred to HFHS and all the paperwork and other conditions required had to be redone.

1. PI joined HFHS on September 1, 2011.

2. Grant was transferred to HFHS on September 30, 2011

3. The required IACUC protocol was approved from the HFHS IACUC committee on Febuaray 22, 2012. The delay was as we missed the October review date, our protocol could be discussed only in the November meeting. Then due to issues of absentee lay person reviewer in the committee, it lagged for another month and the final approval was given to us in February.

4. After which the ACURO form was submitted. The ACURO approval came on March 19, 2012

5. Presented below is the work accomplished since April 2012

BODY:

Effects of diet modulation on body weight and circulating hormones: Dietary modulation was achieved by feeding, 6-7 week old female C57B6 mice (i) regular diet (RD; 10 kcal% fat), ad libitum; (ii) high-energy diet (HED; 60 kcal% fat) ad libitum and (iii) calorie restricted diet (CR; 30% decreased nutritionally supplemented) for 60 days. The HED fed mice started showing a distinct weight gain after 15-20 days and continued to gain weight at a steady rate. The RD and CR followed almost the same rate slow rate of weight gain **(Fig. 1A)**. At end of 60 days, the HED group weighed 20% more than RD and CR, while CR and RD groups had no significant difference. Physiological assessment from the plasma revealed HED mice to have significantly higher levels of glucose, leptin, IGF-1 and insulin, compared to both RD and CR groups **(Figs. 1B,C,E,F)**, while adiponection levels were similar to that of RD. The CR group exhibited significantly lower levels of leptin and IGF-1, with higher level of adiponection compared to RD, while it showed no difference in glucose and insulin levels. These data indicate a successful establishment of generating three separate diet phenotypes, where the negative effects of HED are reflected in weight gain and increased glucose and hormone levels, while the CR mice showed the healthiest profile.

Effects of diet modulation on ovarian tumor growth and progression: To determine the effect of dietary modulation on ovarian tumor growth, C57B6 mice (n=10/group) on the dietary groups of RD; HED and CR as described above were injected with ID-8 mouse tumor cells (ID-8 cells 5 x 10^6 suspended in 100 μl of PBS) intra-peritoneally (IP), post 30 days on diet. After 60 days of tumor cell injections, mice were autopsied for tumor evaluation and specimen collection. As observed earlier, the HED mice gained significant weight, separating it from the RD and CRD groups **(Fig 2A)**, but towards the end of the study, the HED and RD groups begin to converge, probably due to the accumulating ascites in the RD group and muscle loss in the HED group as the tumor growth progressed. The CRD group showed similar weights to that of the RD mice, until the tumor injections (day 30), after which they experienced a sudden weight loss, which eventually stabilized. This sudden weight loss could be due to the appetite loss associated with tumor growth, which is being more apparent in the CRD group **(Fig 2A)**. The average weight for each group at the end of the experiment is shown in **figure2B**. Interestingly RD mice had the highest volume of ascites compared to HED mice, while the CRD mice had the smallest amount of ascites accumulation **(Fig. 2C)**.

Tumor burden was estimated by enumerating the grossly visible tumor nodules on various vital organs **(Fig. 3)**. Scoring was done as 0: no visible nodules; 1: 1-2 nodules; 2: 2-5 nodules and 3: more than 5. The HED mice showed higher tumor burden both in number and size of tumor nodules compared to both RD and CRD groups while CRD mice showed the least tumors. Tumor burden score revealed significantly higher score in kidney, liver and spleen of HED mice compared to the RD mice, while other organs (peritoneum, diaphragm, and bowel) showed no significance, although most mice had a trend towards the higher side **(Fig. 3)**. The CRD group had very significantly decreased tumor burden at all sites compared to the HED group. In comparison to the RD, the CRD mice also exhibited significantly decreased tumor burden at all organ sites, except the peritoneum, which did show a decreasing trend but was not statistically significant **(Fig. 3)**.

Examination of the H&E stained sections of the vital organs corroborated the gross tumor score. Sections from HED mice showed the highest number and size of tumor nodules present in the peritoneum, diaphragm, adipose and lung, compared to RD or CRD groups **(Figs. 4A, B, C, D)**. It was interesting to find tumor nodules metastasized in the lungs as these nodules were not visible to the gross examination. The CRD sections from all organs showed the least number and spread of

tumor growth compared to both groups. The kidney, liver and spleen H&E sections showed tumor nodules associated on the surface only and we could not detect any tumors that had invaded the respective tissue (data not shown). Overall, HED significantly potentiates the tumor growth and metastatic spread, while CR remarkably reduced the tumor growth and spread of ovarian tumors.

Effects of diet modulation on hormone levels: Levels of hormones involved in regulating energy balance like adipokines (leptin and adiponectin), insulin and IGF-1 were estimated in plasma and ascitic fluid, by ELISA. The HED mice showed higher levels of insulin, IGF-1 and leptin in both plasma and ascites, while adiponectin levels unchanged compared to RD group **(Figs. 5Ai, ii; 5Ci, ii and 5Bii)**. The CRD mice had the lowest levels of insulin and IGF-1 in plasma compared to HED and RD **(Fig. 5Ai)**. CRD mice also showed an increase in the adiponectin levels in plasma **(Fig. 5Di)**. In ascites CRD group had lower levels of insulin, IGF-1, leptin compared to HED, while adiponectin levels were unchanged **(Figs. 5Aii, Cii, Dii)**. Comparison between the CRD and RD, showed significant differences in the insulin and leptin levels, where insulin levels were lower and leptin was slightly elevated. Adiponectin levels did not show any significant alteration among the three groups in ascites **(Fig. 5Di, ii)**. These data show that the HED fed mice show the profile of positive energy state while the CR mice exhibit a negative energy profile based on the levels of growth hormones, which correlates with the tumor growth seen in them. Also comparing the CR and RD groups, it can be suggested that the main tumor retardation effects of CRD are due to decreased production of insulin, IGF-1 and leptin.

Effects of diet modulation on cytokine levels: Cytokine (MCP-1, VEGF, and IL-6) levels associated with promoting ovarian cancer were estimated in plasma and ascitic fluids collected from each mouse by ELISA (Fig. 6). The HED mice showed overall increased production of MCP-1, VEGF and IL-6 both in plasma and ascites, compared to RD and CRD, except MCP-1 which did not attain significance in plasma compared to RD **(Fig 6 A,B,C)**. The CRD mice showed significantly lower levels of all cytokines in plasma and ascites compared to HED group. Compared to RD, the CRD group had significantly lower levels of MCP-1 and IL-6 in plasma **(Fig 6 Ai,Ci)**, while VEGF and IL-6 levels were lower in ascites **(Fig 6 Bii,Cli)**. Interestingly, MCP-1 in ascites of CRD showed higher levels than RD group **(Fig. 6Aii)**. These data indicate that diet modulation also affects the cytokine milieu that may contribute to changes in the tumor environment, with HED leading to excess production of pro-tumorigenic factors, while CR restricts them.

Calorie Restricted mice show activation of AMPK pathway:
AMPK is a key nutrient sensor and the master regulator of energy homeostasis in all cells and organisms. Recently, it has been linked to the beneficial effects of CR and that it could be the main mechanism behind CR. To assess if calorie restriction treatment resulted in activation of AMPK, we stained the tumor sections from peritoneum and adipose tissue for p-ACC, the direct downstream target of AMPK. The RD tumors showed some basal expression of p-ACC, which was significantly increased in CRD compared to RD and HFD **(Fig. 7A)**. Further confirmation was done by staining for the downstream targets of AMPK activation. Tumor sections from CR showed a lower expression of p-mTOR **(Fig. 6B)** and p-Akt **(Fig. 6C),** compared to RD and HFD. We did not find a difference in the expression of Ki67 among the three groups (data not shown). These data strongly suggest that calorie restriction results in AMPK activation in tumor tissues and modulates cell cycle and protein synthesis to restrain tumor growth by inhibition of mTOR and Akt.

Pharmacological intervention as calorie restriction mimetic:
We wanted to investigate if the pharmacological activation of AMPK will also result in effects similar to those seen by CRD. For this purpose, we had proposed to use two compounds: metformin and

berberine. Unfortunately, while berebine showed good initial results, the mice begin to have the complication of acute constipation at the dose and schedule we were following. Hence, we had to discontinue the use of berberine and present here the data obtained with use of metformin.

Metformin as calorie restriction mimetic: To observe if metformin treatment can have similar benefits as those of CRD, mice were fed with HED, RD and CRD as before. A group of mice on HED and RD were treated with 100mg/bd kg wt in drinking water daily from the day of tumor injection till the end of the study. Post 60 days of tumor injections, mice were sacrificed and evaluated as explained above. Tumor score indicated that metformin reduced the tumor growth and spread in both the RD and HED groups, but the mice on CRD showed even lower tumor score in peritoneum, diaphragm, spleen, liver and bowel **(Fig. 8)**. The gross scoring was validated by the H&E sections of peritoneum, adipose and diaphragm **(Fig. 9)**. While the tumor score was not significant on all counts, the H&E stains showed metformin treated groups to have less number and size of tumor nodules associated with them. Metformin treated tumor tissue showed higher activation of AMPK, as seen by immuno staining for pACC, the immediate downstream target of AMPK, as did the CRD group **(Fig. 10)**. These data show that metformin treatments also have the ability to limit tumor growth. Metformin seems to be more effective in controlling tumor growth in the background of high energy diet. The ongoing cytokine/growth factor profile of the bio-fluids will reveal if the metformin's effect is similar to CR or has involvement of some additional factors.

ONGOING STUDIES:
1. Targeted gene array of the HED, RD and CRD tumors: We had proposed in the grant to perform, a global gene array to assess the gene based differences in the tumors of various groups. But since then the technology of gene array has been made obsolete by whole genome RNA sequencing, the cost of which is now beyond the budget of the present target. Instead we have opted to performed targeted array for the Insulin-IGF pathways as it is the most prominent pathway being highlighted in the profiles of the various groups. The arrays are being currently performed.
2. Multi-plex cytokine and growth factor profiling of the sera and ascites from the metformin treated group are being carried out to obtain more comprehensive picture of the physioilogical changes occurring
3. Metabolomics profiling: The tumors from HED, RD and CRD and their respective metformin groups are being evaluated. We had previously proposed to do the metabolomics only in the HED, RD and CRD groups and validate the findings in the metformin group. We have changed our strategy now to getting the metabolomics done in all the groups. Since we do not now have the access to a satisfactory metabolomics core, we are getting this done from a commercial company.

Data from these two approaches will be combined as described in the grant.

Limitation: One of the biggest limitations we faced was to obtain enough tumor tissue from the tumors of CRD and the metformin treated groups as the tumors were too small. This also limited our options of the array studies to be performed.
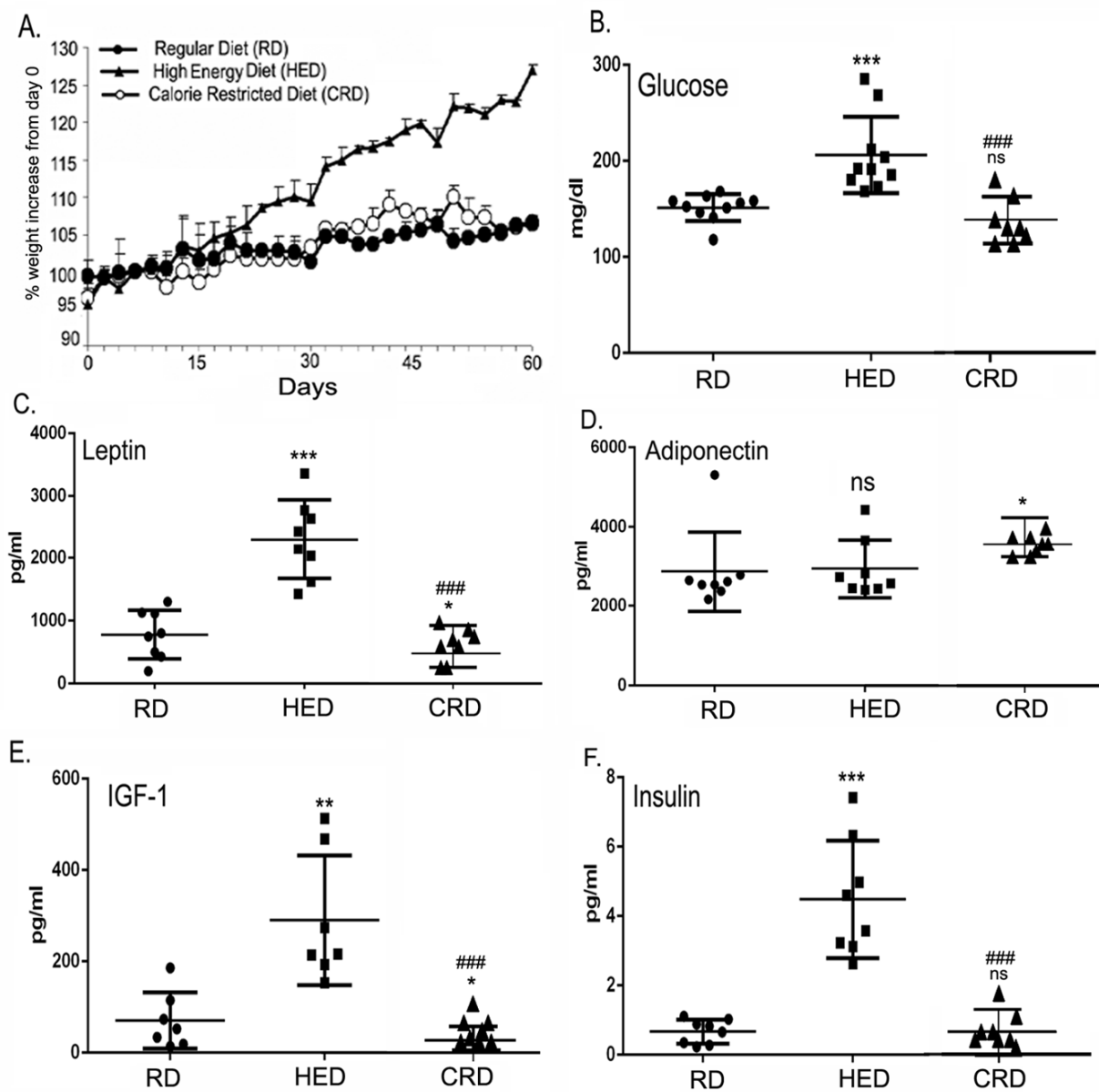
Figure 1: Effects of diet modulation on body weight and circulating hormones: C57B6 mice (n=10) were fed with regular diet (RD) or High energy diet (HED) or 30% calorie restricted diet (CRD). Weight of the mice was measured every alternate day until 8 weeks, after which the mice were sacrificed and blood collected. **(A)** Average weight progression of the mice groups on various diets. HED mice had the highest and the CRD mice had the least levels of glucose **(B)** Leptin **(C)** IGF-1 **(E)** and Insulin **(F)**, while adiponectin levels were not significantly changed in the HED vs RD groups **(D)**. ***$p<0.001$, **$p<0.01$, *$p<0.05$ compared to RD. ###$p<0.001$ compared to HED. ns: non-significant compared to RD.
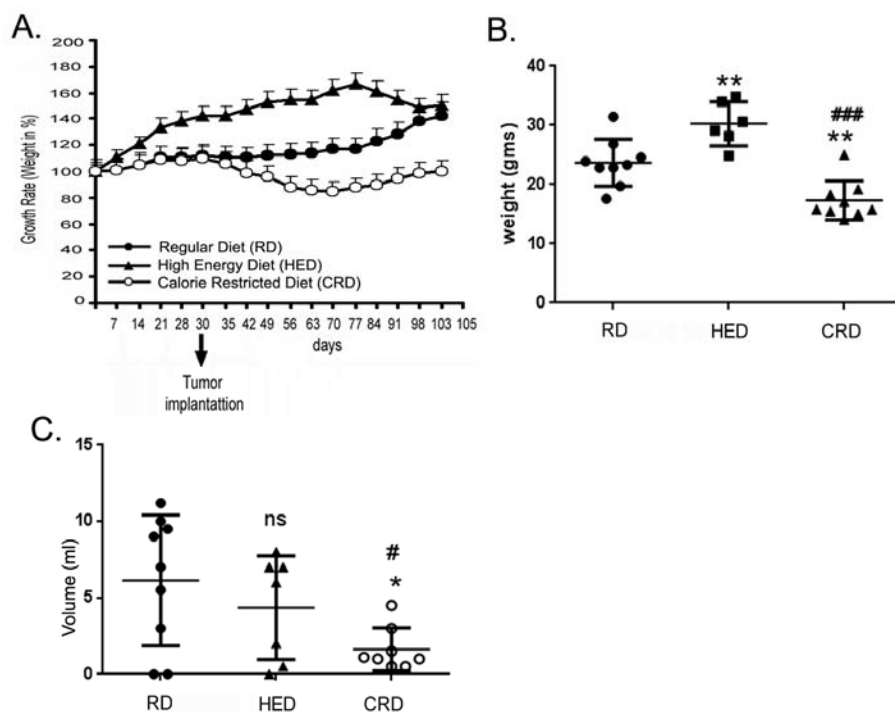
Figure 2: Effects of diet modulation on ovarian tumor growth and progression. (A) Weight progression of the tumor bearing mice in various groups. **(B)** End weight of the mice at the time of sacrifice. **(C)** Ascites volume. **p<0.01, *p<0.05 compared to RD. ###p<0.001, #p<0.05 compared to HED. ns: non-significant compared to RD.
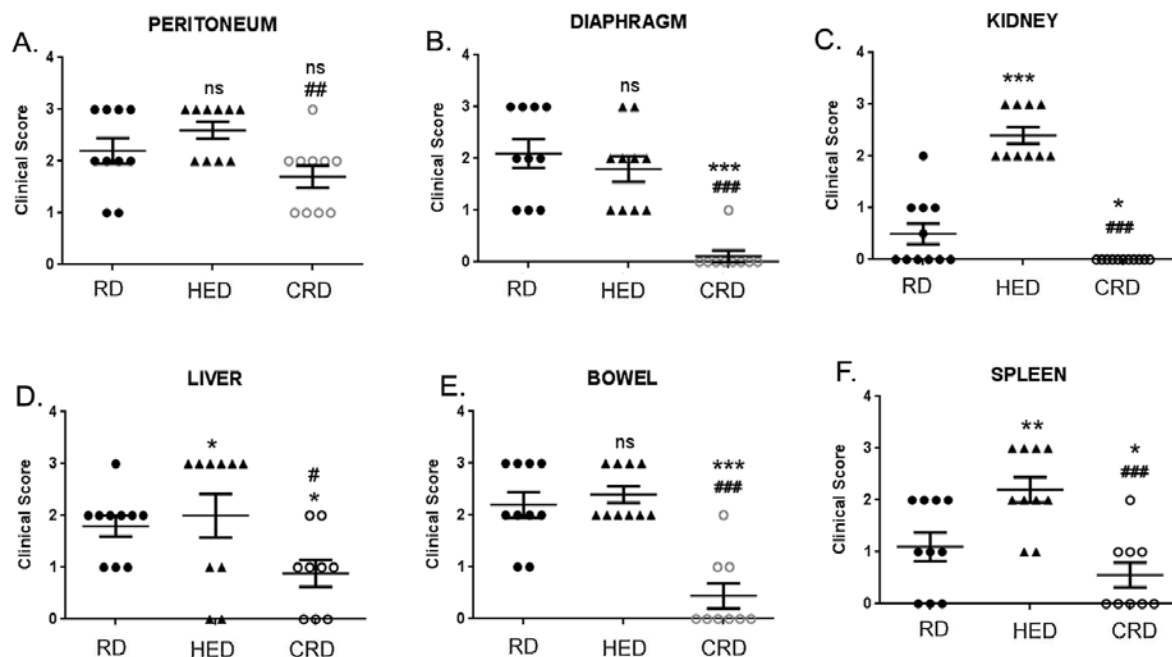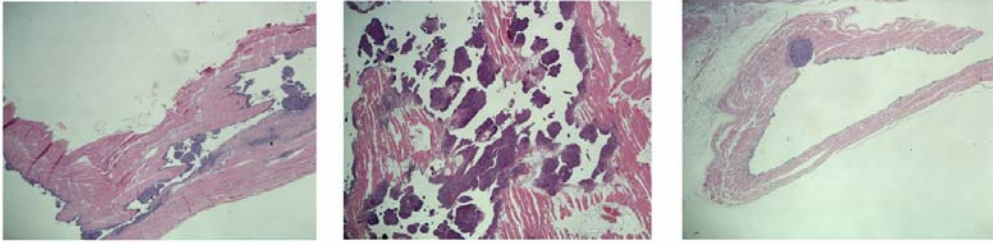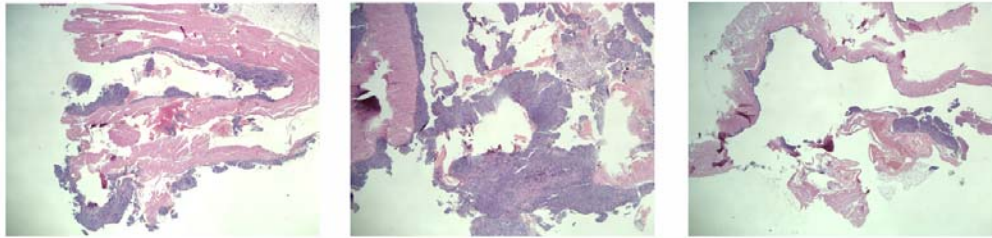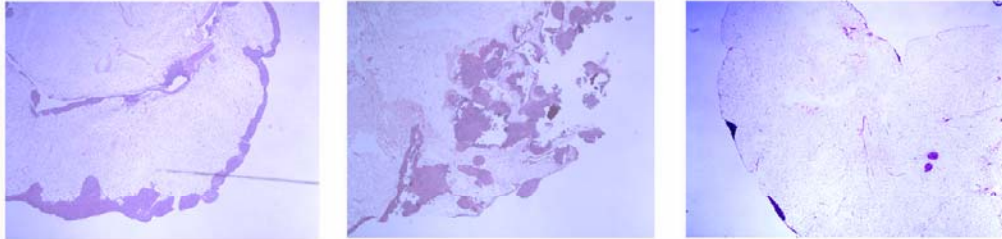

Figure 3: Effects of diet modulation on tumor score. A gross examination of the tumor nodules visible on various organ sites was enumerated using a scoring system where 0: no visible nodules; 1: 1-2 nodules; 2: 2-5 nodules and 3: more than 5. CR had the least amount of tumor burden while HED group had the most tumor counts in the: **(A) peritoneum (B) diaphragm(C) kidney (D) liver (E) bowel and (F) spleen.** The most visible difference between the HED and RD was in the counts of distant organs. ***p<0.01, ***p<0.01, *p<0.05 compared to RD. ###p<0.001, ##p<0.01 compared to HED. ns: non-significant compared to RD.
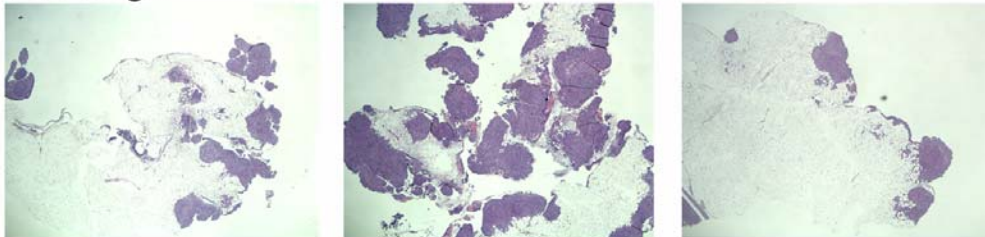
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Figure 4: Effects of diet modulation on tumor burden. A microscopic examination of the tumor growth was performed in the H& E stained tumor sections obtained from various sites of the tumor growth **(A)** peritoneum **(B)** diaphragm**(C)** adipose and **(D)** lung showed that tumors from HED mice were numerous compared to those of RD and CRD, while the CRD had the smallest and the least number of tumor nodules.
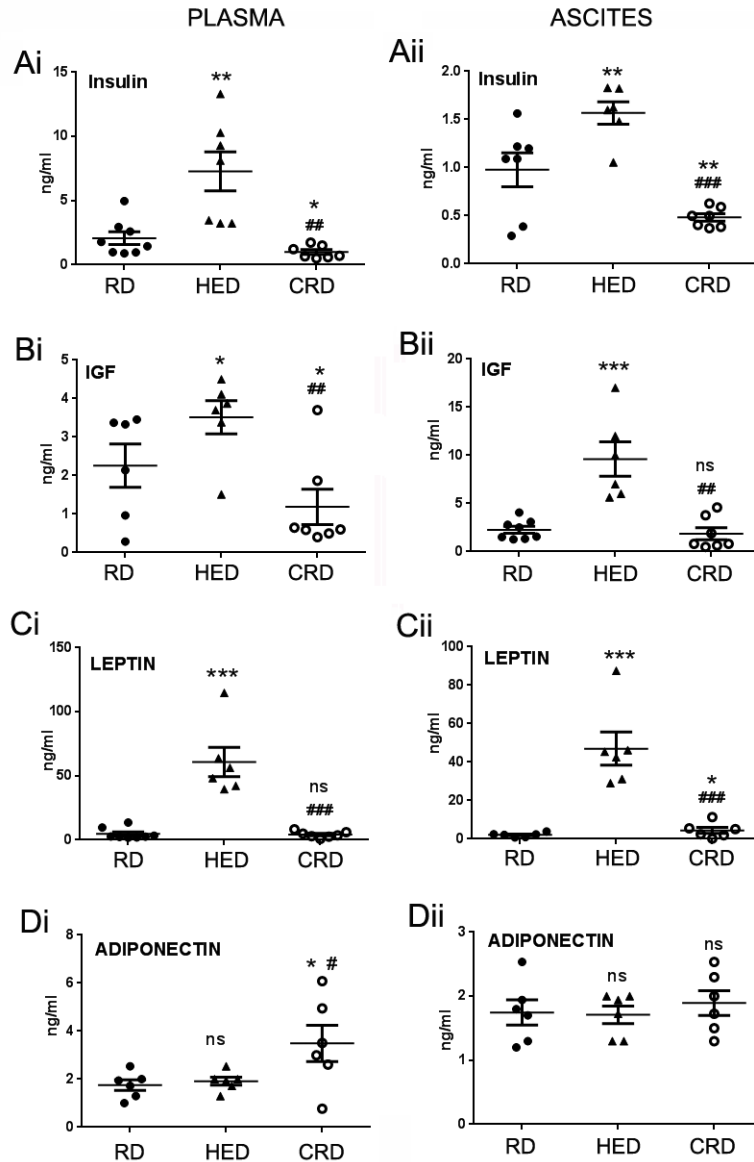
Figure 5: Effects of diet modulation on growth factors and hormones. Plasma separated from blood collected and ascites samples were subjected to ELISA for various growth factors. **(A) Insulin:** HED mice had higher levels of insulin, while CRD had the lowest levels compared to both other groups in plasma (Ai) and ascites (Aii). **(B) IGF:** HED mice had higher levels of IGF, while CRD had the lowest levels compared to RD in plasma (Bi). HED still showed higher levels in the ascites, while CRD levels were similar to RD groups (Bii). **(C) Leptin:** HED mice had higher leptin levels compared to RD and CRD, in the plasma and ascites. (Ci, Cii). CRD levels were non-significant to RD group (Bii). **(D) Adiponectin:** levels did not show significant change, except CRD plasma, that showed elevated levels compared to RD and HED (Di). ***p<0.001, **p<0.01, *p<0.05 compared to RD. ###p<0.001, ##p<0.0,#p<0.05 compared to HED. ns: non-significant compared to RD.
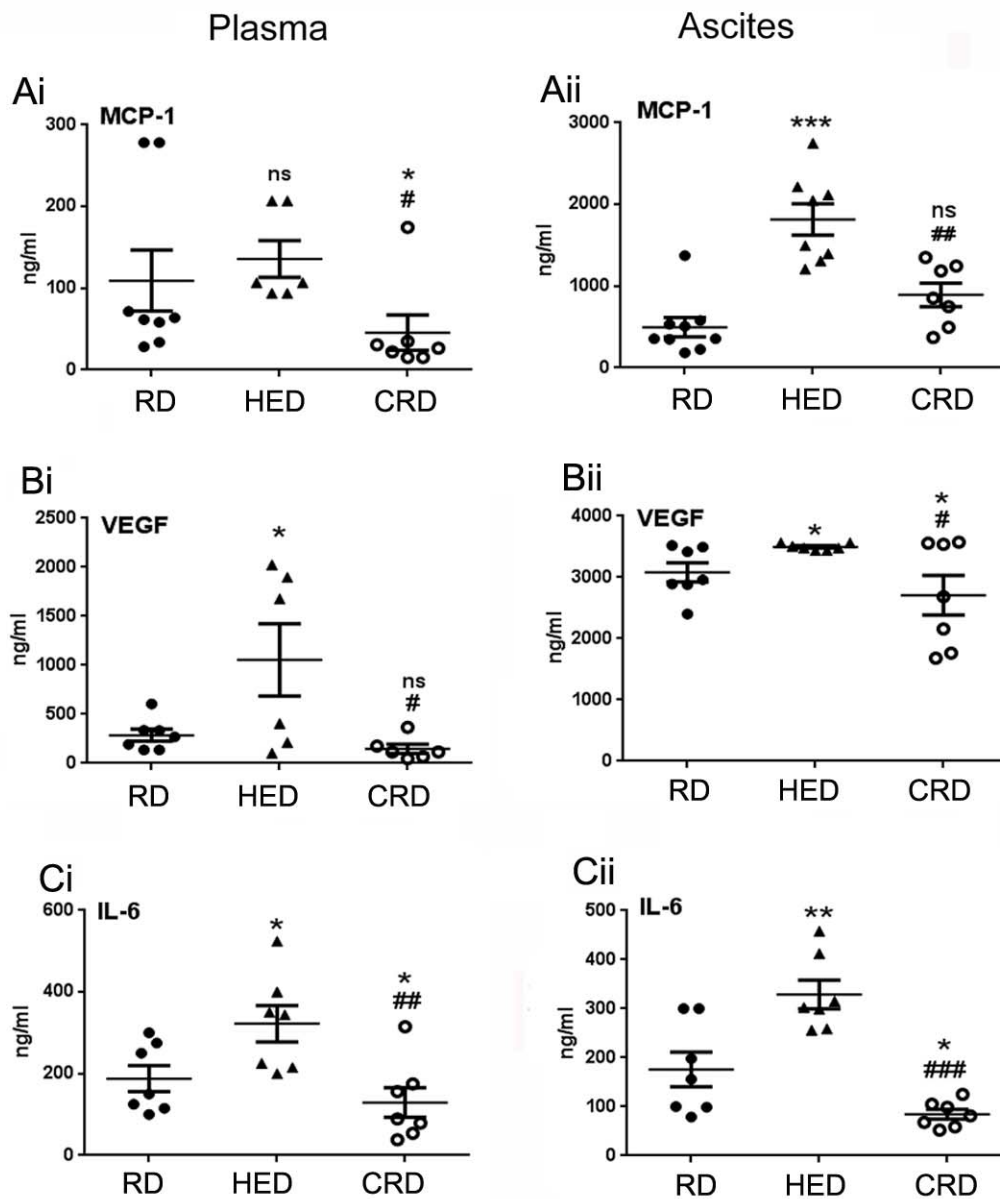
Figure 6: Effects of diet modulation on cytokines. Plasma separated from blood collected and ascites samples were subjected to ELISA for various cytokines. **(A) MCP-1:** HED mice had a non-significant trend towards higher levels of MCP-1, while CRD had the lowest levels compared to both other groups in plasma (Ai) and ascites (Aii). **(B) VEGF:** HED mice had higher levels of VEGF, while CRD had the lowest levels compared to RD in plasma, although the difference between CRD and RD, was not statistically significant (Bi). HED still showed higher levels in the ascites, while CRD levels were the lowest (Bii). **(C) IL-6:** HED mice had higher IL-6 levels compared to RD and CRD, in the plasma and ascites, while the CRD group had the lowest levels. (Ci, Cii). ***p<0.001, **p<0.01, *p<0.05 compared to RD. ###p<0.001, ##p<0.0, #p<0.05 compared to HED. ns: non-significant compared to RD.
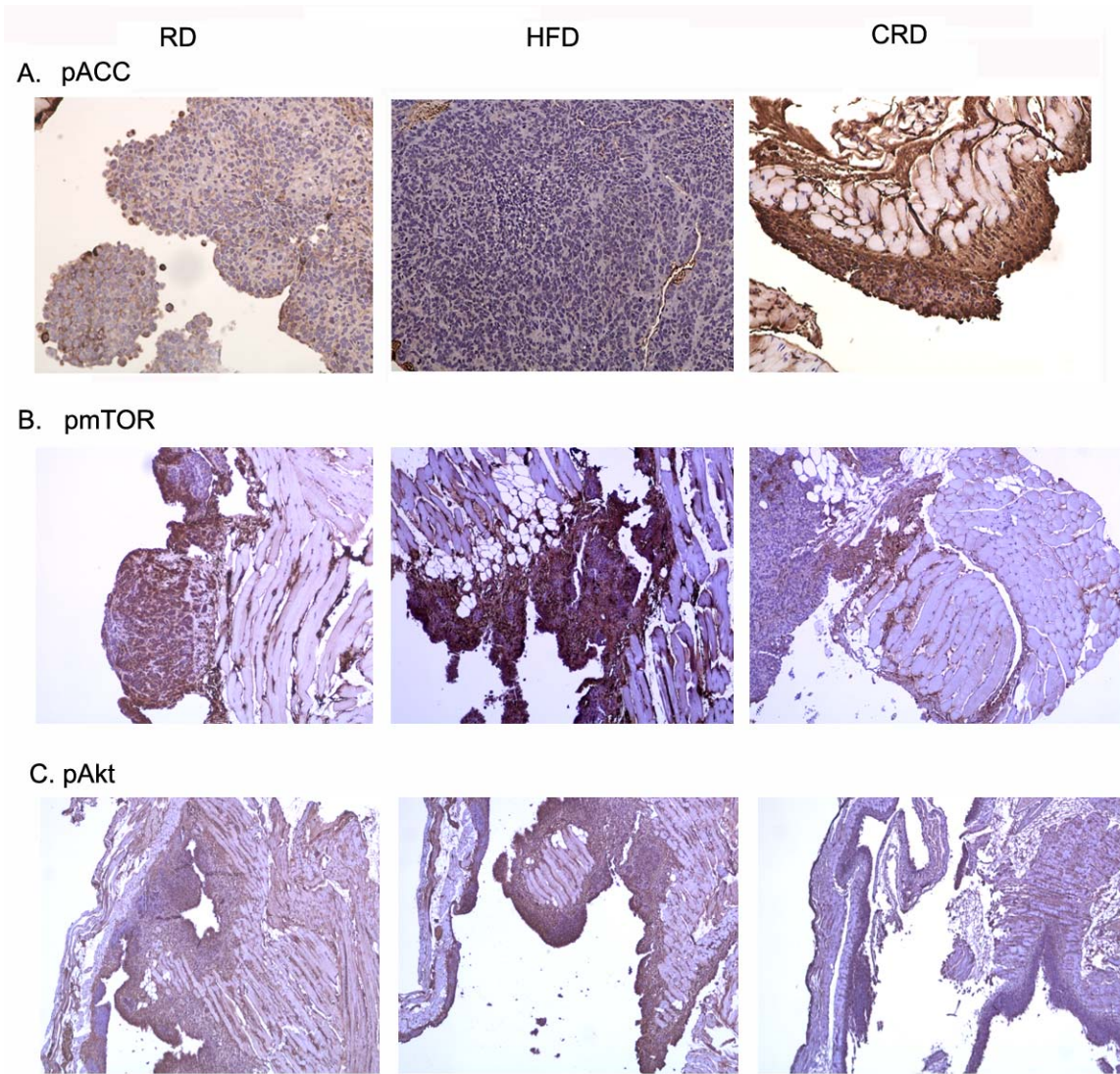
Figure 7: Calorie restricted mice show activation of AMPK pathway: Paraffin tumor sections from the peritoneum sites were immuno-stained for pACC, pmTOR, and pAkt to observe for the activation of the AMPK pathway and its downstream effects. A bright field microscopic examination of the stain was performed. **(A) pACC:** CRD tumors showed a higher positive staining for pACC, HED had the least, while RD group showed some basal positivity. This indicates that a calorie restricted state can induce AMPK activation. **(B) pmTOR and (C) pAkt** stained intensely in the HED tumors, while CRD tumors showed the least positive staining compared to both RD and HED groups, indicating that HED tumors have an activated Akt-mTOR pathway, which appears to be suppressed in CRD tumors. Each stain was performed in 5-6 individual mice tumors from each group.
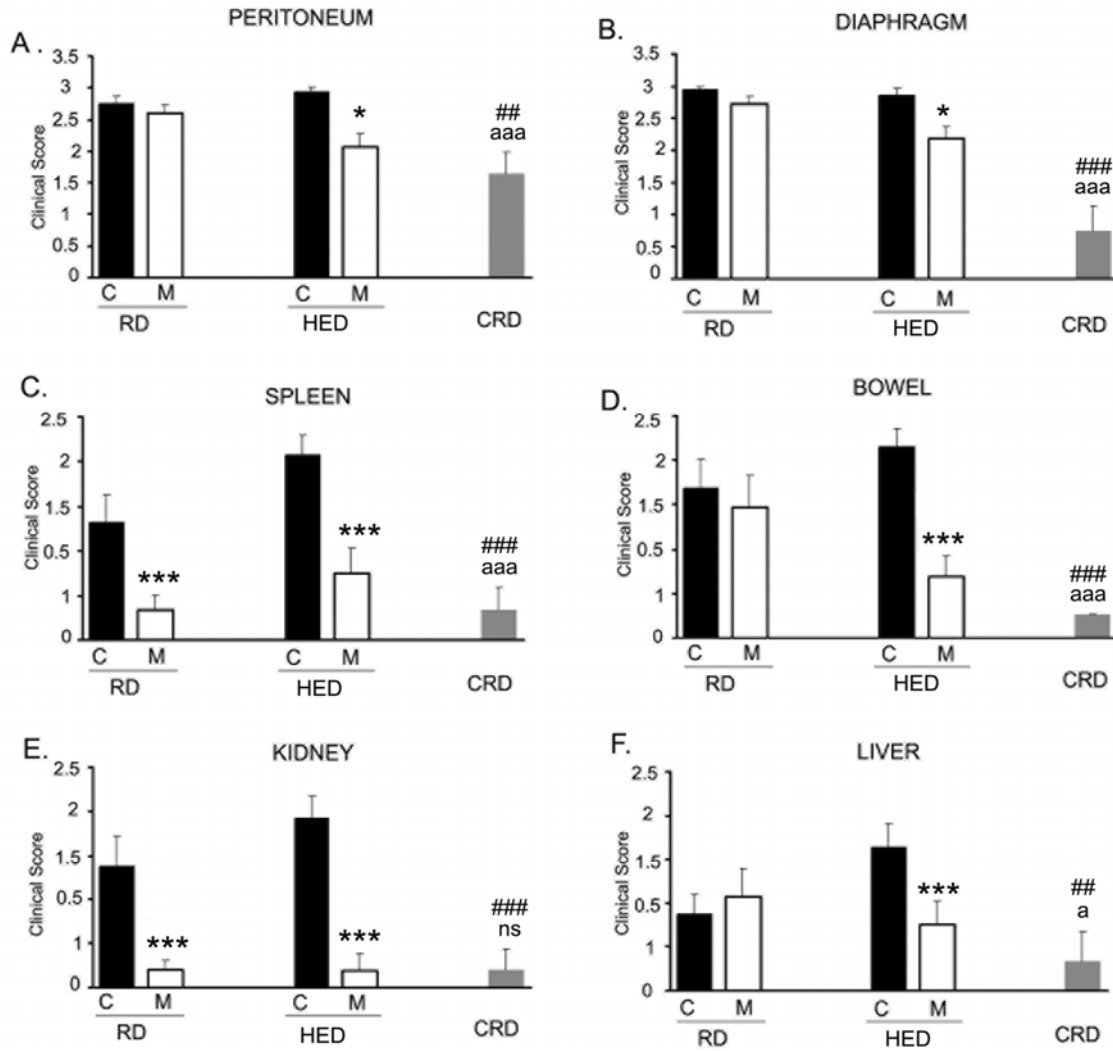
Figure 8: Effect of metformin treatment on tumor score. A gross examination of the tumor nodules visible on various organ sites was enumerated using a scoring system where 0: no visible nodules; 1: 1-2 nodules; 2: 2-5 nodules and 3: more than 5. In the RD set, metformin treated group had decreased tumor burden in (C) spleen and (E) kidney only, indicating its effect more in limiting the spread of ovarian tumors. In the HED set, metformin treatment lowered the tumor burden in **(A)** peritoneum, **(B)** diaphragm, **(C)** spleen, **(D)** bowel, **(E)** kidney and **(F)** liver. CRD mice had the least tumor burden in all the organs compared to all the groups. ***p<0.01, *p<0.05 compared metformin treated compared to their respective untreated groups. ###p<0.001, ##p<0.01 CRD compared to HED and RD. aaa-p<0.01, a-p<0.05, ns: non-significant CRD compared to metformin treated groups.
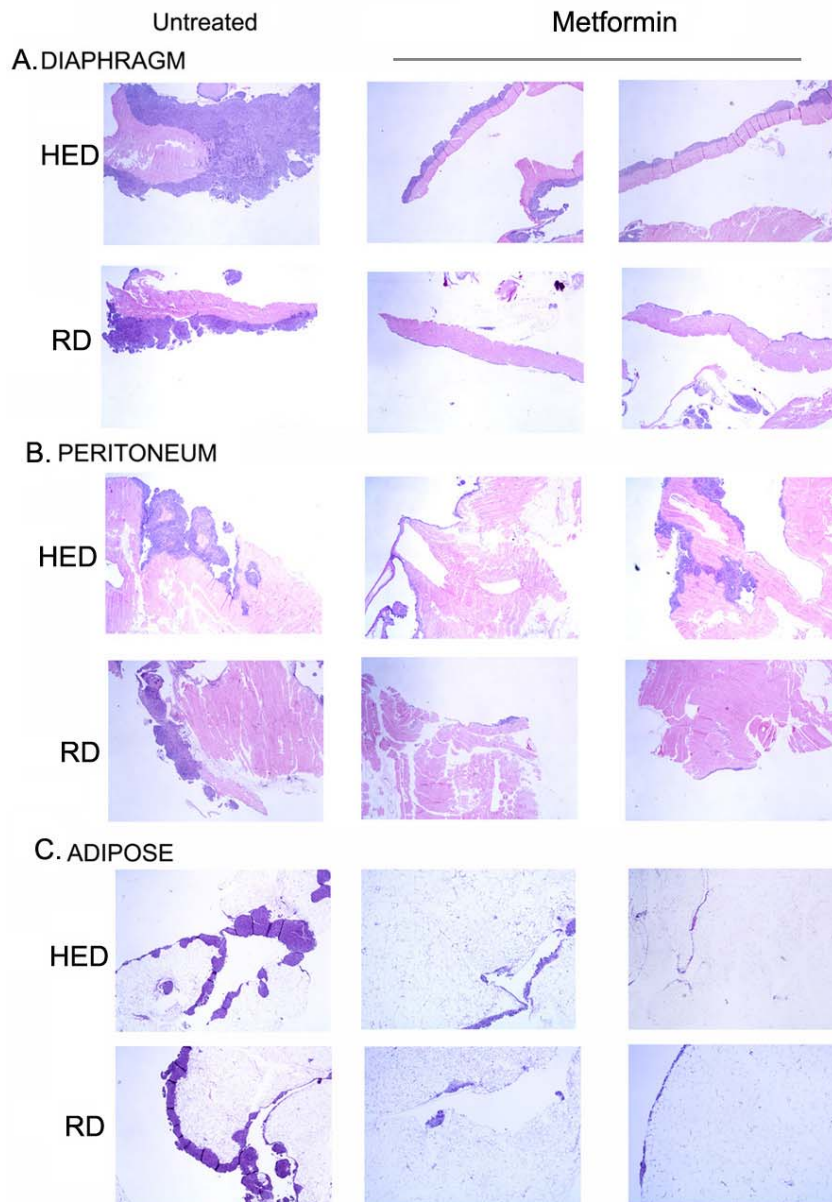
Figure 9: Effects of metformin treatment on tumor burden. A microscopic examination of the tumor growth was performed in the H& E stained tumor sections obtained from various sites of the tumor growth. **(A)** diaphragm **(B)** peritoneum and **(C)** adipose showed that tumors from HED mice were numerous compared to those of RD as seen before (left-most panel). Metformin treatment in both HED and RD groups (two right panels) show decreased tumor nodules and size, indicating inhibition of tumor growth, although the gross tumor burden score did not show such significant changes. These are representative of H&E sections from all mice in the experiment.
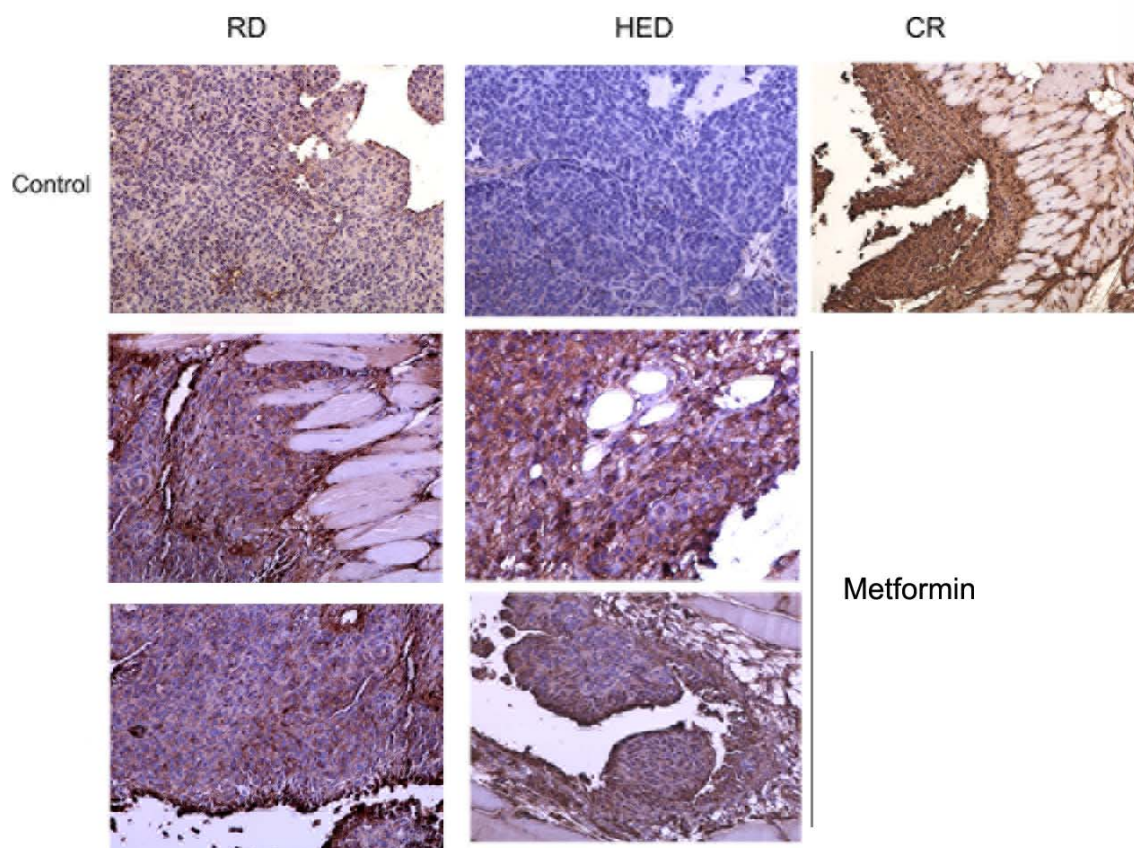
Figure 10: Calorie restricted and metformin treated mice show activation of AMPK: Paraffin tumor sections from the peritoneum sites were immuno-stained for pACC, for the activation of the AMPK. A bright field microscopic examination of the stain was performed. CRD tumors showed a higher positive staining for pACC, HED had the least, while RD group showed some basal positivity as observed in the previous group (top panel). Metformin treatment in both groups (lower 2 left panels) also showed activation of AMPK as seen by positive pACC stain. Each stain was performed in 5-6 individual mice tumors from each group.

Key Research Accomplishments:

1.   Ovarian cancer progresses more aggressively under high fat conditions, while calorie restriction significantly limits the growth.
2.   Calorie Restriction results in robust activation of AMPK pathway and downstream inhibition of Akt-mTOR signaling
3.   Insulin; IGF-1 and Leptin, MCP-1, VEGF may be playing a vital role in influencing ovarian cancer progression.
4.   Metformin treatment is capable of significantly slowing the progress of ovarian tumors under regular or high fat diet conditions.
5.   Effect of metformin treatment is more pronounced under high fat diet conditions.
6.   The most noteworthy change is seen in tumor burden of bowel, spleen, kidney and liver nodules, indicating the inhibition in metastatic spread by metformin treatments.

REPORTABLE OUTCOMES:

1. Ovarian cancer progresses more aggressively under high fat conditions, while calorie restriction significantly limits the growth.
2. Metformin treatment is capable of significantly slowing the progress of ovarian tumors under regular or high fat diet conditions, more so under high fat diet conditions.
3. The most noteworthy change is seen in tumor burden of bowel, spleen, kidney and liver nodules by metformin intake, indicating the inhibition in metastatic spread by metformin treatments.
4. Calorie restriction seems to be the best approach in limiting ovarian cancer growth and spread.

ABSTRACTS:

1. Al-Wahab Z , Tebbe C, Hijaz M, Giri S , Munkarah AR, Rattan R. (May 2013) The Role of Metformin and Diet Modulation in the Progression of Ovarian Cancer. Michigan ACOG Junior Fellow meeting, Lansing, MI. Selected for oral presentation.
2. Al-Wahab Z, Giri S, Munkarah AR, Rattan R. (March 2013) The Role of AMPK in Diet Modulation and Progression of Ovarian Cancer. Wayne State Research Day, Detroit, MI

Both abstracts were selected for oral presentations and won the first category award.

We presently have 2 abstracts submitted to SGO 2014 meeting, about which we will know in November.

DEGREE:

This work will also be a part of thesis for Zaid Al-Wahab, for his Gynecologic Oncology Fellowship, (Wayne State University/Karmanos Cancer Institute) thesis, which will be completed in July 2014.

CONCLUSION

1. Ovarian cancer progresses more aggressively under high fat conditions, while calorie restriction significantly limits the growth.
2. Calorie Restriction results in robust activation of AMPK pathway and downstream inhibition of Akt-mTOR signaling
3. Insulin; IGF-1 and Leptin, MCP-1, VEGF may be playing a vital role in influencing ovarian cancer progression.
4. Metformin treatment is capable of significantly slowing the progress of ovarian tumors under regular or high fat diet conditions.
5. Effect of metformin treatment is more pronounced under high fat diet conditions.
6. The most noteworthy change is seen in tumor burden of bowel, spleen, kidney and liver nodules, indicating the inhibition in metastatic spread by metformin treatments.
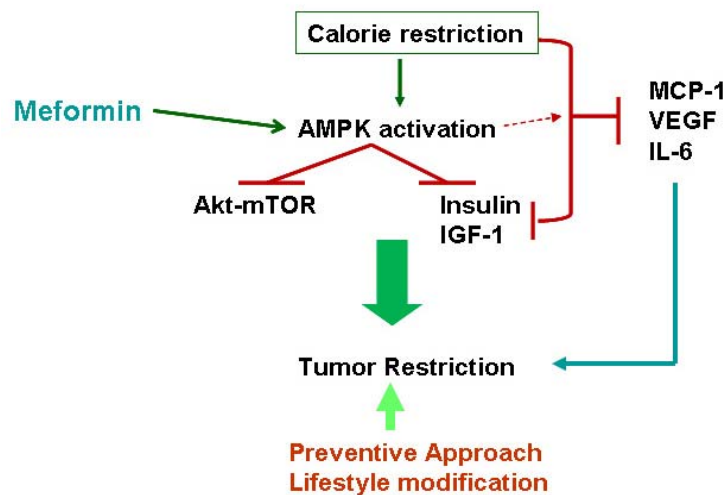

Figure 11: Overall picture: Calorie restriction by activation of AMPK can lead to inhibition of Akt-mTOR pathway and growth factors like insulin and IGF-1, which work together to restrict the ovarian tumor growth. Calorie restriction also leads to lower levels on inflammatory cytokines like MCP-1, IL-6 and angiogenic VEGF, which also contribute to inhibition of tumor growth. Weather these are also under AMPK regulation is not yet clear. Metformin can mimic calorie restriction and can bring about similar downstream changes. This opens a new new avenue for ovarian cancer where calorie restriction or pharmacological mimetics can be introduced as preventive or life style changes that contribute to decreased ovarian cancer growth.

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1. Ramandeep Rattan, PhD: PI
2. Calvin Tebbe, BS: Lab Technician
3. Jasdeep Chhina, BS: Lab Technician (replaced Calvin Tebbe in July 2013)

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